different pattern sizes and variations in the microstructure, respectively. Here we report on the advantages of PDMS-alternatives for an improved and fast micro-contact printing process. Using those materials we could print proteins in various structures (including pillars, lines and grids) and sizes down to 100 nm. For characterization of pattern quality we used TIRF and super-resolution microscopy and focused on the validation of well-known protein-protein interactions including the one of EGF-, Insulin/IGF1- or beta-adrenergic receptors with intracellular binding partners. As TIRF microscopy requires homogenously adhered cells on the micro-patterned surface, we especially focused on the verification of cell adhesion efficiency when using different pattern shapes.
An important issue which we are currently addressing is the production of a micro-structured and functionalized multiwell plate (96 and 384 well design). This development step will set a milestone concerning the throughput rates of the micro-patterning assay and increase the number of potential users interested in this methodology.

2425-Pos Board B562
Millisecond Time Resolved Electrochemical Detection of Non-Electroactive Neurotransmitter Release
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Acetylcholine and glutamate are highly important non-electroactive neurotransmitter in the mammalian central nervous system. A fast, sensitive method to detect the release of acetylcholine and glutamate at the surface of a single cell is needed to gather data about the kinetics of exocytosis events in pathways involving these signaling molecules.
To this end, carbon fiber electrodes have been modified with electrodeposited gold nanoparticles to increase the effective electrode surface area and provide a high curvature surface for enzyme attachment. For detection of acetylcholine, acetylcholine esterase and choline oxidase were deposited onto the nanoparticle coated electrode surfaces to catalyze acetylcholine to hydrogen peroxide for electrochemical detection. The functionalized electrodes have been characterized to determine the KM and Vmax of the enzymes as well as the total enzyme coverage and gold nanoparticle surface area. This information was further used to evaluate the conditions for optimal retained enzyme activity of the sensor surface. Similarly, glutamate oxidize was placed onto the surface of electrodes plated with nanoparticles. The sensors were tested for acetylcholine and glutamate release from a synthetic cell model for exocytosis, and providing time resolved detection of single vesicle release events on the order of millisecond time scale.

2426-Pos Board B563
Solid-State Nanopore Detection of Epigenetic DNA Modifications
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We describe protein-facilitated solid-state nanopore detection of dsDNA containing single modified nucleotides. We first use model mono-biotinylated oligonucleotides to determine the detection limits of the assay by systematically studying the effect of DNA length and biotin position to better understand the detection process. We then investigate epigenetic modifications by combining selective biotinylation of target modified bases and a charged, high-affinity protein tag to induce translocation events. We use this approach to resolve two major epigenetic modifications: methylcytosine (5mC) and hydroxymethylcytosine (5hmC).

2427-Pos Board B564
Dynamics and Energy Contributions for Transport of Pertactin through an Aerolysin Nanopore
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Autotransporters are a large family of extracellular monomeric virulence proteins from Gram-negative bacteria. Despite their simplicity, many aspects of the autotransporter secretion mechanism remain unclear. We are using pertactin, an archetypical autotransporter from Bordetella pertussis, as a model for secretion studies. The final step of autotransporter secretion is C-to-N-terminal transport of the central passenger domain through the outer membrane, mediated by the C-terminal translocator domain. Passenger folding occurs only after this final secretion step, which requires neither ATP nor a proton gradient. Passenger folding may therefore serve as a driving force for pertactin secretion. For this reason, it is interesting to consider how autotransporters are secreted through their own translocator domain to the cell surface.
As a first step, we are mimicking this transport using a simpler model consisting of a well-known nanopore. Transport of the pertactin passenger is detected at the single molecule level using electrophysiological techniques. We show that unfolded pertactin dynamics through a single aerolysin pore can be described using a model developed for an unrelated protein. A Van't Hoff-Arrhenius law describes the frequency of blockades as a function of the applied voltage. The unfolded chains are dominated by an activation energy that has both an entropic component and an enthalpic origin. We compare our experimental results to theory and show that proteins cross the membrane by passing through the aerolysin nanopore. We have used these results to develop a general description of the comportment of an unfolded protein during its transport through a protein nanopore.

2428-Pos Board B565
A New Environmental Biosensor for Cell Free Synthetic Biological Systems
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Biosensors utilize fundamental properties of biophysics to enable detection of environmental analytes. While these systems are frequently based on living cells, they have potential applications in multiple fields. Cell-free synthetic biological systems, such as artificial cells (i.e., liposomal encapsulations of functional biological parts), are an example of systems that could be enhanced by new biosensors. Here, we created a biosensor based on the biophysical interactions of biotin and streptavidin. Biotin plays an essential role in cell growth and the typical amount of biotin required by cells is low (e.g., 1 ng/ml in *E. coli*). Biotin is also widely used in molecular assembly because of its strong conjugation to streptavidin, with a K_d around 10^{-15} M). We leveraged this strong attraction in a competitive binding scheme to create a biotin sensor that is both specific and sensitive in comparison to common biotin assay methods that are based on radioactive labeling, microbiological, or physicochemical principles. This new biosensor has a detection limit in pg range, and significantly discriminates between biotin and its metabolic precursor in *E. coli*, dethiobiotin. This engineered biosensor can be used as a biotin detector for biotin synthesis by engineered cells. Additionally, it can be deployed in cell-free synthetic systems. Ultimately, our engineered biosensor can be coupled to cell-free systems to act as an environmental reporter. Alternatively, it can trigger cell-free gene expression in artificial cells. We anticipate this new technology will impact work in fields ranging from synthetic biology to the biophysics of biomaterial assembly.
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2429-Pos Board B566
Nanopore Sequencing of "Alien" DNA Bases
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Recently, it was shown for the first time that Escherichia coli can efficiently propagate a genetic alphabet expanded from the four canonical DNA nucleotides to include the synthetic ''alien'' bases d5SICS and dNaM. Traditional sequencing platforms cannot detect these alien bases. We tested whether nanopore sequencing with the protein pore Mycobacterium smegmatis porin A (MspA) is sensitive to d5SICS and dNaM. In nanopore sequencing, a nanometer scale pore provides the only electrical connection between two electrolyte solutions. An applied voltage causes a current to flow through the pore. Negatively-charged single stranded DNA is drawn through the pore, causing a nucleotide specific reduction in the measured current. Thus-far, MspA is the only nanopore shown to demonstrate single-nucleotide sensitivity to standard nucleotides as well as methyl cytosine and hydroxymethyl cytosine. We use the φ29 DNA polymerase to regulate DNA motion to single-nucleotide steps. Here, we demonstrate the direct detection d5SICS and dNaM with MspA-enabled nanopore sequencing.

2430-Pos Board B567
Robust Membrane-Embedded phi29 Motor Channel for Sensing of Single Molecule and High-Throughput Fingerprinting of DNA
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The ingenious design of the bacteriophage phi29 DNA packaging motor with an elegant channel has inspired its applications in nanotechnology. The hub of the motor is a truncated cone shaped connector consisting of twelve protein subunits that form a ring with a central 3.6-nm channel that acts as a path for